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SUBUNIT COORDINATION IN THE FIREFLY LIGHT ORGAN[1]

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An interesting type of cellular integration is the synchronous coordination of responses of many effector units to produce a brief pulse which is the summation of the activities of the individual units. Some neuroeffector activities, such as the discharge of electroplaques of electric fish (Albe-Fessard, 1961) are remarkably well synchronized; the duration of activity of the entire organ is comparable to that of a single unit.

Another effector system which exhibits coordination of single units is the firefly light organ. In the genus *Photuris*, there are two light organs located on the ventral portions of abdominal segments 6 and 7, each of which contains about 1000 light emitting cells ("photocytes"). These are grouped into organizational units of 10–16 photocytes in a rosette pattern around a central core ("cylinder") which contains the trachea and nerves. One to four "rosettes" may be stacked vertically through the thickness of the organ. Nerve trunks from segmental ganglia branch to each cylinder and terminate in complex "end organs" near the photocytes (see Buck, 1948; and Smith, 1963; for further histological details).

Neural control of light production has been amply demonstrated (Buck and Case, 1961; Case and Buck, 1963; Buck, Case, Hanson, 1963; and Magni, 1967). Data from localized stimulation of small portions of the light organ (Hanson, 1962) suggest that the physiological unit of control peripheral to the ganglion may be the portion of the light organ supplied by a nerve branch. Visual observations by Buck (1966) indicate that under some experimental conditions, responses can be elicited from smaller units, perhaps the rosettes and individual photocytes. Thus, some ambiguity exists as to the size of the smallest physiological unit which is coordinated in a flash of the light organ.

The normal degree of unit synchrony in the firefly organ is such that the onset of activity appears simultaneous and uniform over the entire surface when observed by the gross methods of low power microscopy or photomultiplier viewing of several limited portions of the tissue (Buck, Case, and Hanson, 1963). As Buck (1966) points out, however, it has not yet been determined whether a whole-organ flash consists of well-synchronized subunit responses, each having kinetics similar to the organ flash or whether it is an integration of staggered briefer responses of the individual units. If the latter is indicated, as the data of Buck (1966) suggest, then identification of the single unit and an analysis of the spatial and temporal distribution of unit responses will add to present knowledge of the excitation mechanism of firefly luminescence.

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With image intensification tubes (Reynolds, 1968), it has become possible to follow the events in the firefly flash using cinematographic techniques with substantial optical magnification. Consequently, we undertook the following study to obtain further information concerning the participation and coordination of unit responses in the firefly effector system.

Materials and Methods

The fireflies used were adults of *Photuris* sp. (probably *versicolor* or *lucicrescens*) collected near Princeton, New Jersey or raised from larvae collected near Bethesda, Maryland.

The firefly was placed in a slot milled into a lucite microscope slide and secured dorsal side down. Restrained in this manner, the firefly flashed either spontaneously or after mild irritation. For some experiments, all or part of the light organ was divested of cuticle by cutting along the edges of the segment and peeling away the cuticle with sharpened forceps. A transient glow emanated from the exposed area. This glow was prolonged for photography by injection of norepinephrine into the abdomen. Scintillation was induced in some animals by abdominal injection of 10^{-3} *M* eserine, an anticholinesterase (*cf.* Case and Buck, 1963).

The slide holding the firefly was placed on the stage of a compound microscope from which the ocular lens had been removed. Light from the firefly was then projected in focus on to an image intensifier tube (English Electric Valve Co., type P-839) operated at gains of 10^3 to 10^6. Using this system, cinematography was possible at up to 64 frames/sec where exposures of 5–10 sec would have

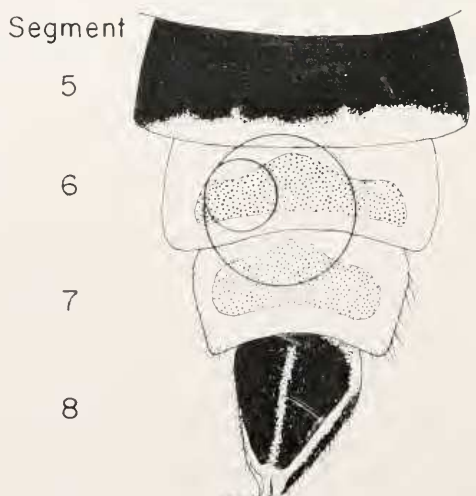

FIGURE 1. Ventral view of the posterior abdomen of a female *Photuris sp.* locating approximately the image intensifier fields for Figure 2 (large circle) and Figure 3 (small circle). Fields shown in Figures 4 and 7 are of the size but not necessarily the location illustrated by small circle.

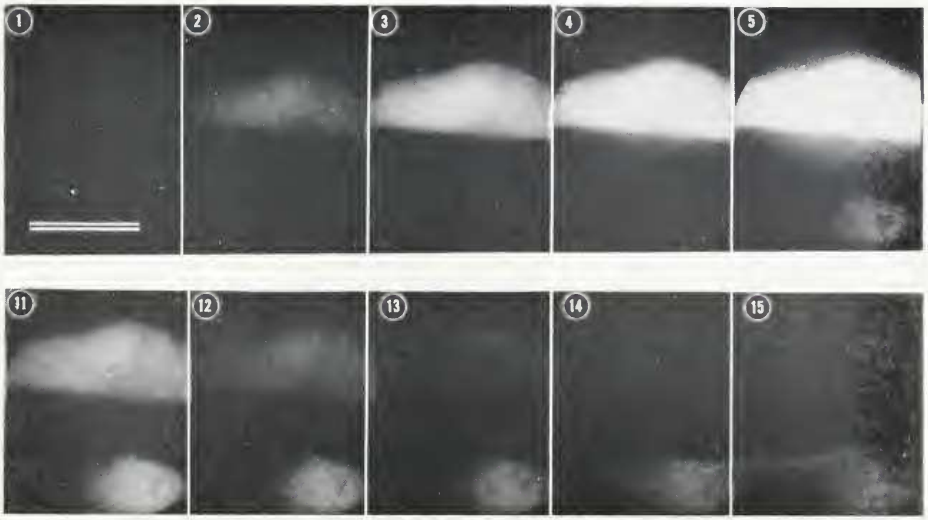

FIGURE 2. Coordination of the two segmental light organs. The two segments sometimes light asynchronously, as illustrated here. Time between frames is 16 ± 2 msec. Only frames 1–5 and 11–15 of a 17-frame flash are shown. The anterior organ turns on 2–3 frames (32–48 msec) ahead of the posterior. Calibration bar = 1 mm. For orientation of the field of the firefly, see Figure 1 (large circle).

been required using conventional optics (Reynolds, 1968). The image tube has an input cathode one inch in diameter and the electron optics result in a magnification of approximately 0.95. The tube is capable of resolving 25 line pairs per millimeter at the input. The dark current (or noise) introduced by the intensifier consists mainly of single electrons emitted from the cathode surface, which result in bright spots of light approximately 40 microns in diameter on the output phosphor. However, about 5% of the noise spots are significantly larger than this and result from multiple electron emissions from the cathode due to ion bombardment. Thus, when the signal consists of nearly circular spots, it is important that optical magnification in the system occur before the intensifier cathode. In the present work this magnification (3×, 20×, and 100×) was accomplished by the objectives of the microscope.

The anode was photographed with a 16 mm Bolex movie camera at 32 or 64 frames/sec. Calibration of the shutter cycle of the camera indicated considerable variability; however, periods of constant shutter speed occurred when the camera was neither accelerating after being turned on nor decelerating as the spring neared its unwound limit. In such periods the cycling interval was 16 ± 2 msec at "64 frames/sec" and 32 ± 4 msec at "32 frames/sec." All records were selected from such periods of uniform shutter speed in the middle of a run.

The positions of the image intensifier field relative to the light organ of the firefly at the lower magnifications are illustrated in Figure 1. Several types of luminescent activity were photographed; normal flashing, dim flashing, scintillation, and glowing. For dim flashes and for scintillation, enlarged positives were made from the cinefilm and the spots of light from the firefly organ were identified

and traced from frame to frame on a transparent overlay. Thus, the appearance of each spot could be followed as the flash waxed and waned. In the dim flashes, the spots were followed from flash to flash as well as from frame to frame. A long sequence of submaximal flashes was filmed from one animal, but only those in best focus (flash numbers 1–3 and 17–19) were analyzed. The interval between these two series was about two minutes.

To determine the sizes of the microsources, the diameter of each spot in flashes 17–19 was measured. Calibration was accomplished using photographs of a micrometer slide mounted in place of the firefly and viewed through the same optical system.

The sizes of the photocytes, cylinders, and rosettes were determined from photographs of standard histological sections of *Photuris* sp. cut 10 μ thick in an orientation parallel to the ventral surface. Tissues were fixed in Navaschin fixative and stained with Delafield's hematoxalin and eosin. These photographs were supplied by John Buck and Miriam McLean of the National Institutes of Health (see acknowledgments).

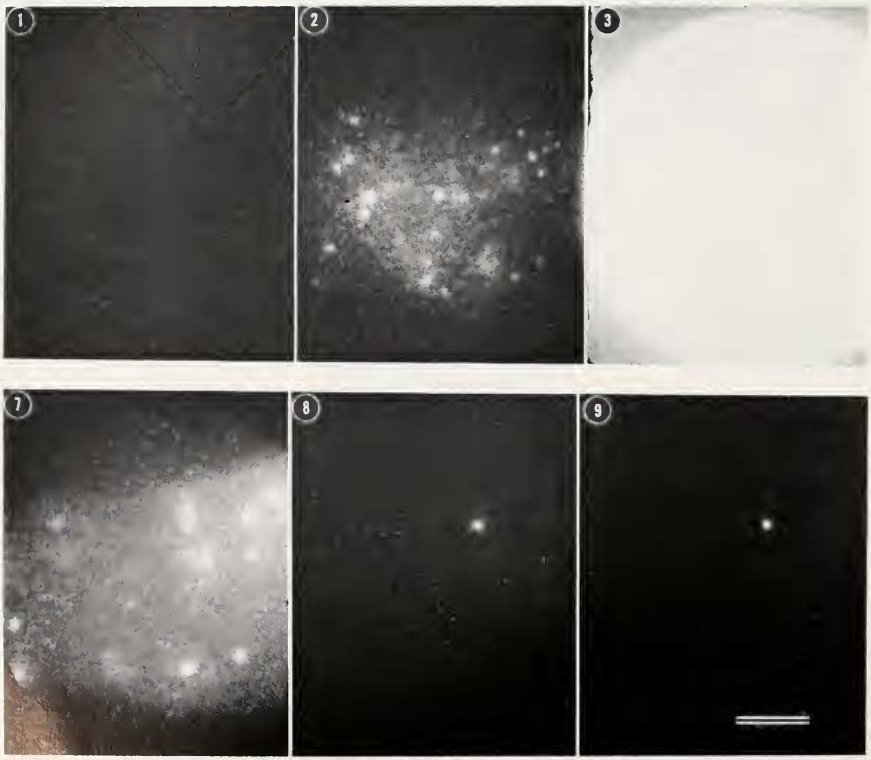

FIGURE 3. Coordination of microsources in a normal flash. Some sources precede and others lag the rest of the organ. Only frames 1–3 and 7–9 of an 11-frame flash are shown. Frame 3 is overexposed due to the flash of the entire organ. Three frames identical to frame No. 3 have been omitted between rows. Somwhat more than $\frac{1}{3}$ of the sixth-segment's light organ is shown (see Fig. 1, small circle). Calibration bar is 200 μ. Time between frames is 32 ± 4 msec.

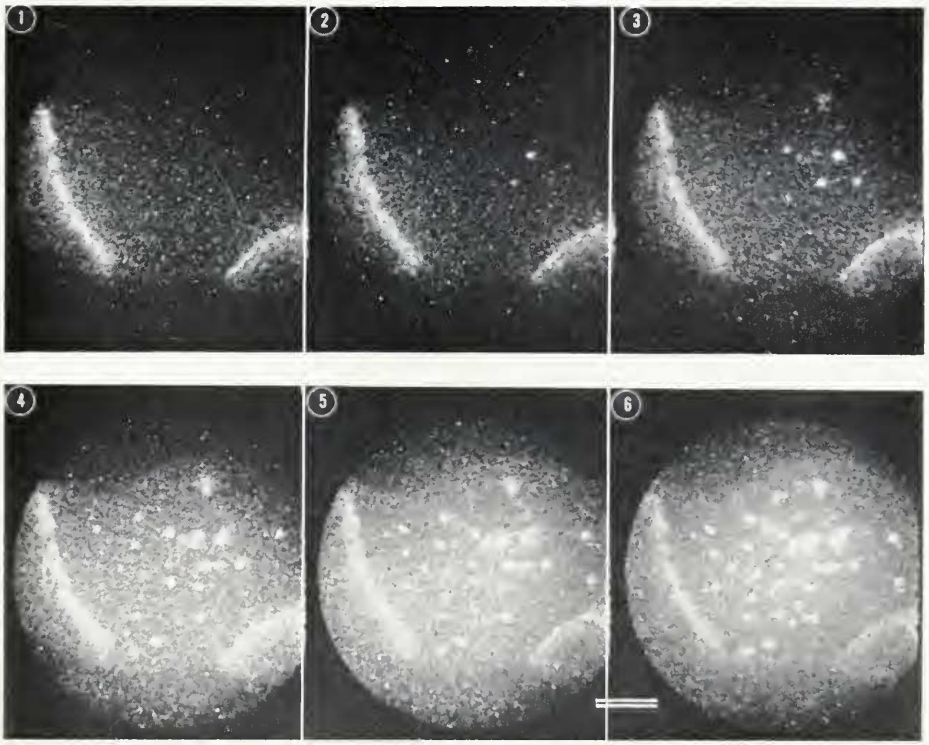

FIGURE 4. Coordination of microsources in a submaximal flash. Frames 1–6 of flash No. 18 in a series of filmed submaximal flashes. Note that spots remain in constant relative positions through several frames. Diagonal light bands are tissues glowing because of incisions through the cuticle overlying the light organs. These bands serve as markers for registration of the transparent overlay used in identifying spots on successive frames. The differences between tube noise spots and microsource spots are easily observed in frames 1 and 2. Time between frames is 16 ± 2 msec. Calibration bar is 200 μ.

Where temporal information was not required, superimposition prints were prepared by sequentially projecting several frames of the negative cinema film on the same photographic paper at reduced intensities which eliminated most of the noise spots. This procedure was followed for Figures 8, 9, 10b, 12, and 13.

RESULTS

I. *Coordination of gross areas of organs*

The extent of temporal coordination between the two light organs of the firefly has been examined with the image intensifier system. Results show that usually both organs flash coincidentally (within 16 msec); however, occasionally the anterior or posterior segment may lead. Figure 2 shows a filmstrip taken at 64 frames per second with low optical magnification in which the anterior segment leads the posterior segment by 2–3 frames, or 32–48 msec (see Fig. 1 for orientation of the field on the firefly).

Lack of complete synchrony can also be seen within a single organ. In Figure 3, individual spots (frame 2) precede the activity of the rest of the organ (frame 3). The decay phase is also asynchronous, with small areas of activity lingering on well past the extinction of the others.

II. *Coordination of Microsources*

Normally when an organ flash is observed, the details of single unit activity are not seen due to lack of contrast. However, fireflies occasionally emit submaximal flashes consisting of small spots of light scattered across the otherwise dark organ (Fig. 4). These microsources are of nearly uniform size and appear similar in size and shape to the spots which lead and lag the organ flash (Fig. 3). Thus, microsources appear to be functional units and are here tentatively proposed as the smallest such physiological unit of the firefly light organ.

TABLE 1

Microsource durations

Type of light emission	N	Mean duration of microsources ± S.E.	Range	Organ flash duration (approx.)
*Individual flashes:				
Flash 2	101	151 ± 6 msec	60–340 msec	340 msec
Flash 3	55	156 ± 8	31–360	400
Flash 17	85	147 ± 7	31–310	425
Flash 18	80	112 ± 6	47–250	300
Flash 19	109	124 ± 5	31–250	320
**Pooled flashes	430	139 ± 3	31–360	
***Scintillations	129	81 ± 2	31–156	

* Listed are the durations of the microsources as well as of the total flashes which they comprise. N is the number of microsources. Organ flash durations are only approximate, since the end points were estimated by comparison with a normal flash form.

** Microsource data from flashes 2, 3, 17, 18, and 19 were lumped for these calculations.

*** Scintillation data were obtained from 160 consecutive frames of cinema film. All statistics and ranges were calculated from original data and do not include uncertainties of absolute time calibrations, which is approximately ± 15% (see Materials and Methods).

Spatial and temporal distributions of the microsources within a single flash. Assuming that submaximal flashes are essentially the same as normal flashes except for a reduction in number of participating microsources, cinematographic recordings of the light organ during this reduced activity provide information concerning the participation of unit sources in the organ flash. A record taken at 64 frames/sec demonstrates asynchrony of microsources (Fig. 4). The temporal patterns provided by the sequential appearances of the spot do not correspond to gross morphological structures, such as innervation (Hanson, 1962). However, the spatial distribution of the spots results in a distinct pattern which allows individual sources to be identified in successive frames throughout a flash as well as in successive flashes.

The temporal distribution of microsources throughout a flash is illustrated in Figure 5. This figure shows that individual spots do not remain lit for as long as does the overall organ flash, and that the duration of the latter is partly the result of asynchronous initiation of the former.

The durations of activity of the microsources vary from 31–360 msec (S. D. = 61) and are normally distributed about a mean of 139 msec (see Table I).

Only rarely did a spot appear to extinguish and then revive within a single flash. Only five such cases out of 430 occurred, with an average of 32 msec "off-time." These data suggest that a long recovery period is not required for

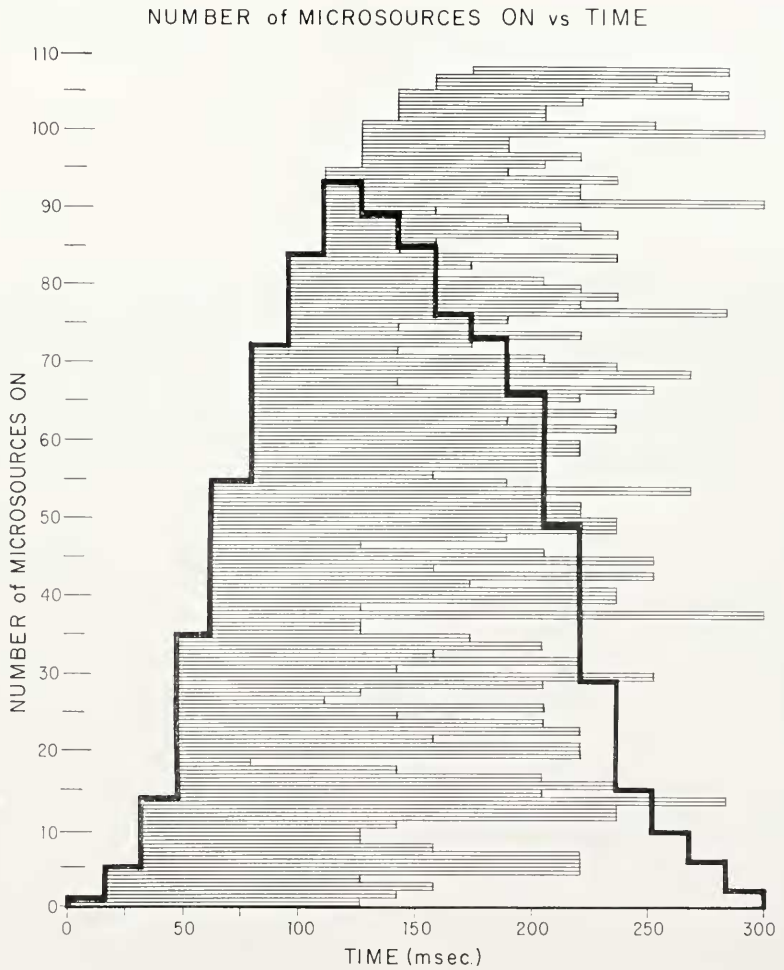

FIGURE 5. Microsource durations in a single flash (No. 19). Each microsource numbered on the ordinate according to order of appearance is represented as a bar (consisting of a line and two spaces) stretching from the time of its appearance to the time of extinction. Vertical steps at left illustrate the number of new microsources appearing in successive frames. The heavy line indicates the total number of microsources visible at any one time.

TABLE 11

Decrement of microsource lifetimes during the course of a flash

	Fraction of flash		
	First $\frac{1}{3}$	Second $\frac{1}{3}$	Final $\frac{1}{3}$
* Mean duration ± S.E.	173 ± 5 (msec)	143 ± 4	101 ± 4
N	145	144	141
P of difference	<0.001	<0.001	

* The total number of spots measured in 5 submaximal flashes (see Table 1) were categorized according to the fraction of the flash in which it was first observed. The means of the durations of all spots in each category were than calculated.

these microsources, or that the microsource is a compound source. Since the light organ has depth, the latter is a possibility.

Spatial and temporal distributions of microsources in successive flashes The spatial pattern of lighting microsources is well reproduced in successive flashes: of the spots which are active in one flash, an average of 76% also appear in the next flash.

The relative temporal order in which the microsources appear in successive flashes also tends to repeat; that is, the order of appearance of the individual spots in four out of five flashes correlated significantly with the order of appearance of the succeeding flashes. This is also reflected in the uniformity of the latencies

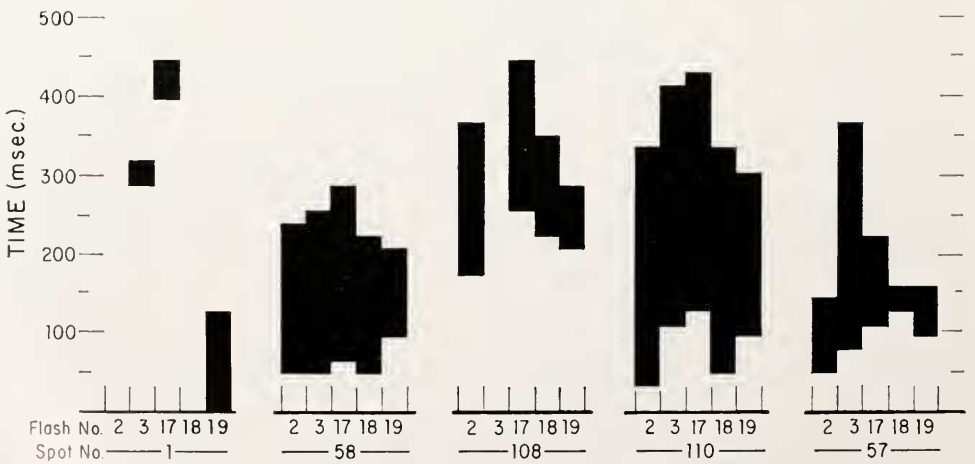

FIGURE 6. Microsource durations in successive flashes *vs.* time after appearance of first microsource. The vertical bars represent the active period of a microsource relative to the beginning of the flash. Horizontal repetition of bars represents the activity of the same microsource in the flashes numbered on the abscissa. The spots shown were specifically selected to illustrate variations in the following response parameters: delay relative to earliest microsource, duration, and dependability of appearance. For example, spot No. 1 appeared only in flash Nos. 3, 17, and 19 with a great deal of variability in delay and duration, whereas No. 58 appeared in all flashes and was quite consistent.

(time between initiation of organ flash and onset of spot activity) from flash to flash of four of the five individual microsources depicted in Figure 6.

Another temporal relationship observed is that between the duration of a microsource and its time of appearance. The correlation in this case is significantly negative ($r = -0.48$, $p < 0.001$), indicating that the earlier spots last longer than those appearing later. This is also illustrated by Table II: responses appearing in the first $\frac{1}{3}$ of the flash last longer than those which comprise the remaining portions of the flash.

III. *Participation of microsources in scintillation*

Scintillation consists of apparently asynchronous activity of minute areas of the light organ which occurs occasionally in normal animals and can be evoked artificially by injection of neurally active drugs, such as eserine. Serial photographs of the light organ during the eserine-induced scintillation shown in Figure 7 provide an indication of this type of activity. The spots of light are similar in appearance to the microsources, and data were obtained in the same manner as in the dim flashes.

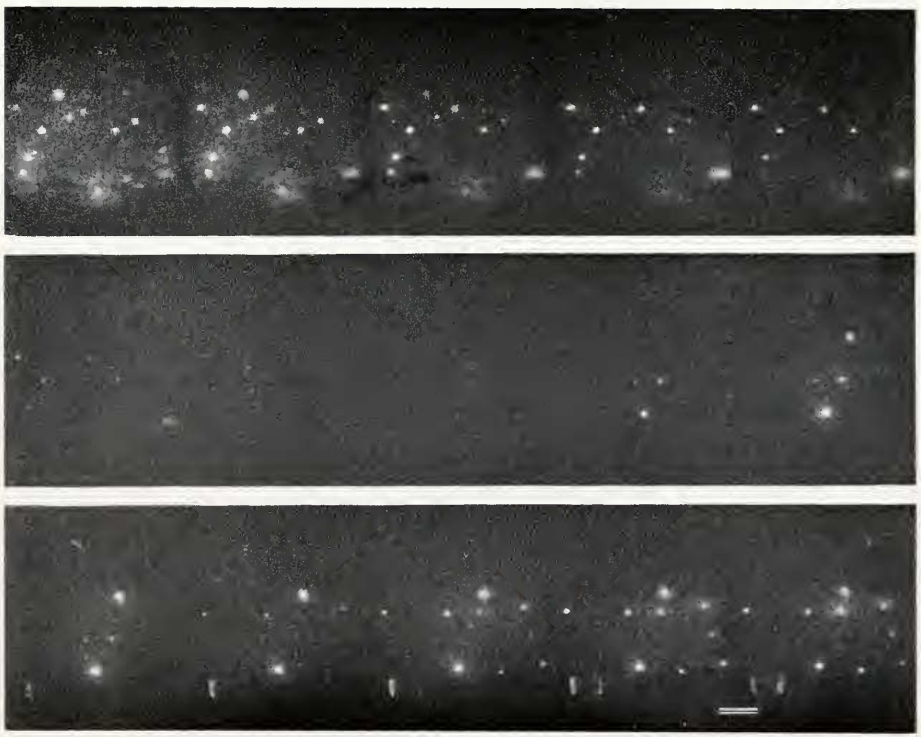

FIGURE 7. Scintillation. A series of 15 consecutive cinema frames taken shortly after injection of 10^{-3} *M* eserine into the abdomen. Note that spots stay in register for several frames. Entire circular field represents about $\frac{1}{3}$ of one segment (see Fig. 1). Time between frames is 16 ± 2 msec. Calibration bar is 200 μ.

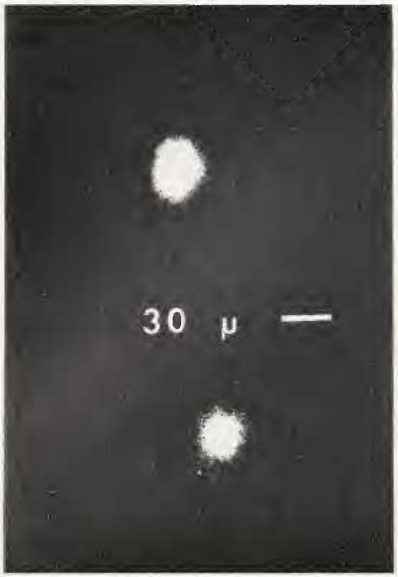

FIGURE 8. Size and shape of individual microsources. The upper is slightly elliptical, 39×30 μ. The lower is nearly circular with a diameter of 26 μ. This figure is a highly enlarged print of a frame from a scintillation series.

The durations of scintillation spots are less than those of flash spots, averaging 81 msec (range 31–156 msec, S.D. = 25, see Table I). No repeatable spatial patterns of the spots were observed.

When observing eserine-induced scintillation, the visual impression is of random activity and it has been described as such in the literature. However, statistical treatment of the temporal data (autocorrelation and Fourier analysis) from one animal showed significant ($p < 0.001$) periods of increased microsource activity at 170 and 1370 msec intervals.

IV. *Shape and size of the microsource*

The usual microsource spot appears round in the photographs (Figs. 4, 7, and 8) although about 10% were ellipses having major: minor axis ratios of 1.3–2.2. Occasional spots are larger and very elongate, and thus are probably aggregates of microsources (Fig. 9).

The sizes of the spots composing submaximal flashes range from 11 to 36 μ with a mean diameter of 21.6 ± 0.3 μ (S.E.), as documented in Table III. The scintillation spots were similar in size, averaging 20.7 μ (range 10–36 μ). The pre- and post-flash spots also fall into this range, averaging 31 μ in diameter. The sizes of the spots were not significantly different from flash to flash, or from flashes to scintillation (Table III).

The diameter of a microsource appears to be quite constant throughout its lifetime, *i.e.*, over the several (average = 8.9) continuous cinema frames on which it is visible. The sizes of the spots in the first and last frames during an appearance

TABLE III

Apparent microsource size during flashes and scintillation

	Number of measurable spots	Mean* diameter ± S.E.	Range
Flash 17	63	21.6 ± 0.8 μ	11–36 μ
Flash 18	70	22.1 ± 0.5 μ	15–34 μ
Flash 19	63	21.0 ± 0.5 μ	15–30 μ
Lumped 17, 18, 19	196	21.6 ± 0.3 μ	11–36 μ
Scintillation	123	20.7 ± 0.4 μ	10–36 μ

* Mean diameters of microsources were determined by measuring the spots in every frame in which a spot appeared. No significant differences between means were detected between any categories.

are 8% smaller than the mean size, but this could be a photographic artifact due to the changing brightness.

DISCUSSION

Identification of the unit source

The foregoing descriptions of the activities of unit sources are independent of their anatomical identity. However, in view of the extensive data on microsource diameter (Table III), such an identification can now be made.

To aid in such identification, a tangential histological section of a light organ is illustrated together with an image intensfied photo of a glowing organ in Figure 10 and a composite diagram of histological features and dimensions (Fig. 11). The histological section (Fig. 10a) shows that the photocytes fan

FIGURE 9. Aggregation of microsources. From the same scintillation series as Figure 8. Such aggregations comprised less than 1% of the scintillation spots.

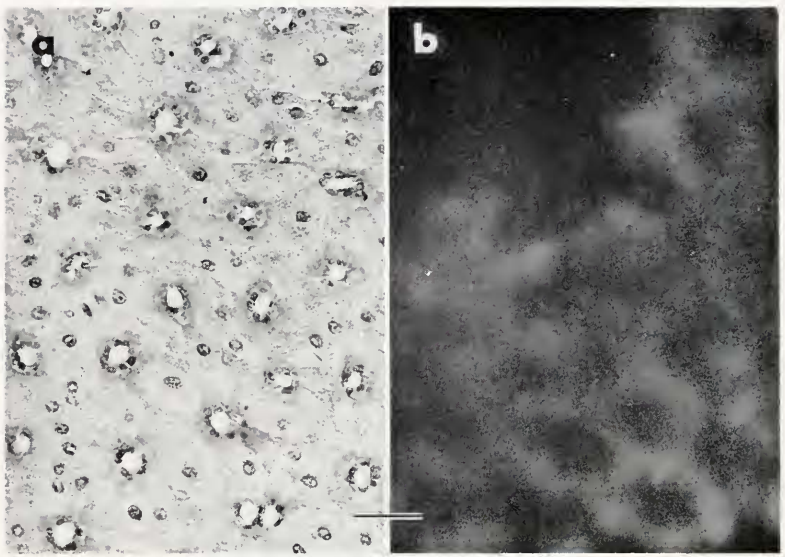

FIGURE 10. (a): Histological section of the light organ of *Photuris*, supplied by M. McLean (see acknowledgments). Tangential section (parallel with ventral surface of the light organ). Open circles *ca.* 10 μ in diameter are trachea cut in cross section; dense bodies immediately surrounding them are the "tracheal end organs" (tracheal end cells, tracheolar cell bodies, nerve endings) as well as the packed mitochrondria in the cortical zone portion of the photocytes adjacent to the tracheal end organs. The "cylinders" average 14 μ in diameter and comprise trachea and end organs. The photocytes radiate out from the cylinders, stretching the entire distance, an average of 34 μ, from the rim of one cylinder to the rim of its nearest neighbor. The darkly stained nuclei of many photocytes appear in this section midway between cylinders. Tracheoles emerging from the tracheal end organs are seen coursing between photocytes in upper right. Center-to-center distances between nearest cylinders range from 30 to 75 μ. (b): Glowing organ at same magnification as 10a. The nearest spacing of dark centers averages 50 μ (range 35–60 μ), which is comparable to cylinder nearest spacing in 10a. Calibration bar is 50 μ.

out in a rosette pattern from the axial "cylinders", which comprise trachea (open circles) plus the tracheal end organs (tracheal end cells, tracheolar cell bodies, nerve endings) seen here as darkly stained nuclei immediately surrounding the trachea. Measurements from several such sections indicate that the average spacing between neighboring cylinders is 42 μ (range 30–75 μ) which compares favorably with 52 μ (range 35–60 μ) for the spacing of the dark centers of the glowing rings in Figure 10b. Thus from geometrical considerations it is clear that the portions of the cylinder which emit light are the more centrifugal elements, namely the photocytes.

Further, the dimensional data illustrate that only part of any photocyte emits light; the width of the bright band in Figure 10b (average: 25μ, range: 20–31 μ) is less than the length of the photocytes in Figure 10a (average: 34 μ, range: 23–50 μ). Furthermore, the dark centers of the glowing rings in Figure 10b appear larger (diameter 25–40 μ) than the diameter of the cylinders (10–17μ) in Figure 10a. Therefore, the dark centers must include the cylinder plus some adjacent portion of the photocytes. The non-luminous portion of the

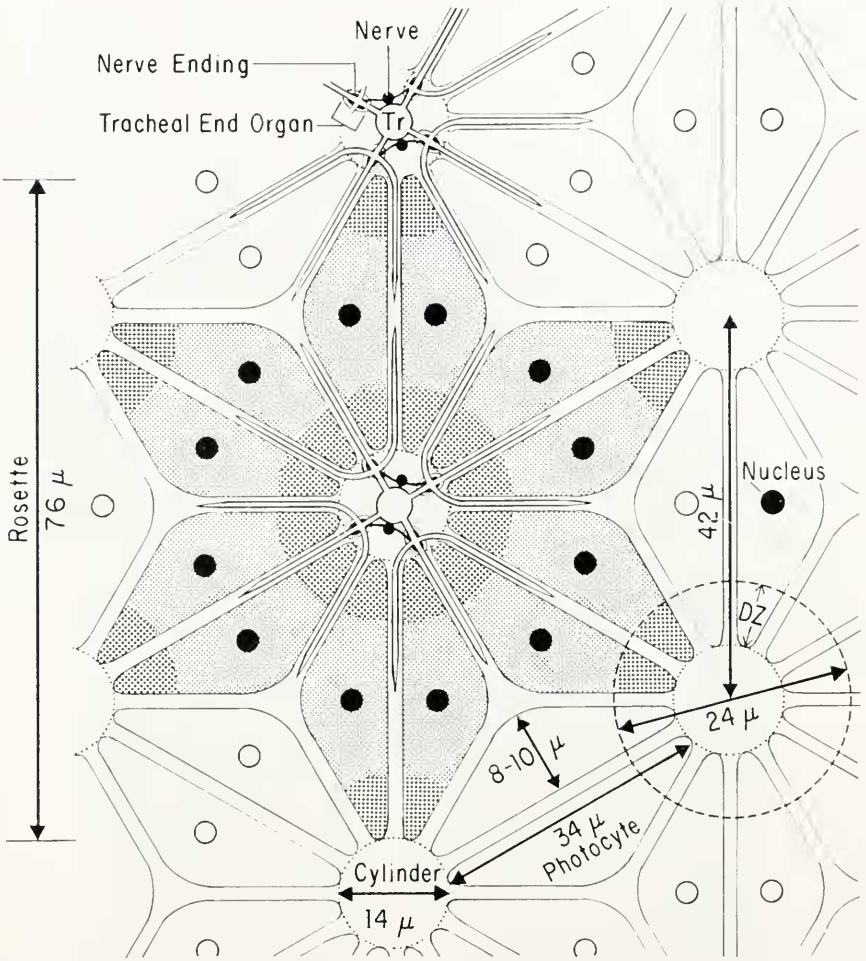

FIGURE 11. Composite diagram drawn from Figure 10a; dimensions refer to means of measurements and thus may not be strictly additive.

photocyte would correspond well with the "differentiated zone" of the peripheral cytoplasm of the photocytes, which lacks the "photocyte granules" so abundant in the central region (see review by Buck, 1948; Beams and Anderson, 1955; Smith, 1963).

It is also evident in Figure 10a and 11 that the diameter of a full rosette of photocytes would be the length of two photocytes plus the cylinder, or 82 μ. Measurements of several such rosettes range from 60–90 μ with an average of 76 μ. The sizes of full glowing rings seen in Figure 10b would be expected to correspond with this anatomical rosette minus the differentiated zones, or about 66 μ. Such measurements of glowing rings average 72 μ in diameter.

Figure 12 is a higher magnification of a portion of a skinned organ showing

two complete glowing rosettes. This figure more graphically illustrates the dark centers 25–40 μ in diameter, surrounded by glowing elipses 15–30 μ long and 7–9 μ wide, some of which are separated by a thin dark line (arrows).

Further evidence that the microsource is smaller than the rosette is seen in Figure 13, in which glowing rosettes and single flash spots of a submaximal flash occur simultaneously in the same organ.

In the above context, the unit source which produces the flashspots and scintillation spots can now be identified. The average diameter of this microsource (21.6 μ) is somewhat less than the average length of a photocyte (34 μ) but is within the width (20–31 μ) of the light band surrounding the dark cylinders in Figure 10b. If only the central portion of a photocyte emits light, as mentioned earlier, a 21.6 μ spot could be accounted for. However, since the width of the photocyte is 6–10 μ, activity confined to a single cell would result in an elongate spot with a major : minor axis rato of from 2 to 5. Yet, only about 10% of the microsources were observed as ellipses, with elongation ratios of 1.3 to 2.2 (average 1.6).

In view of the foregoing, a composite picture emerges which identifies the functional unit source as being the *central portions of two or three adjacent photocytes.*

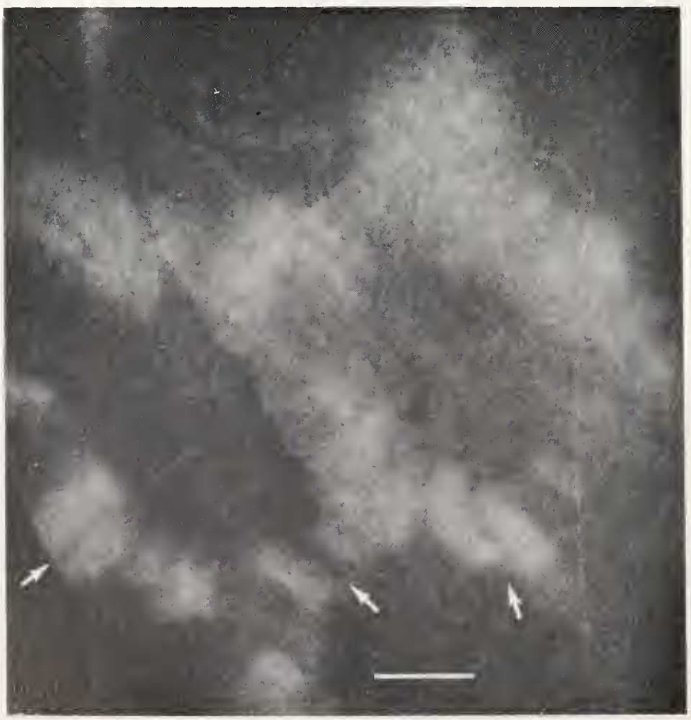

FIGURE 12. High magnification of a glowing light organ. Two complete rosettes are shown along with portions of adjacent ones. A number of light emitting areas which are about the size and shape of individual photocytes (7×21 μ) can be seen, with some pairs of photocytes separated by a thin dark line (arrows). Calibration bar is 20 μ.

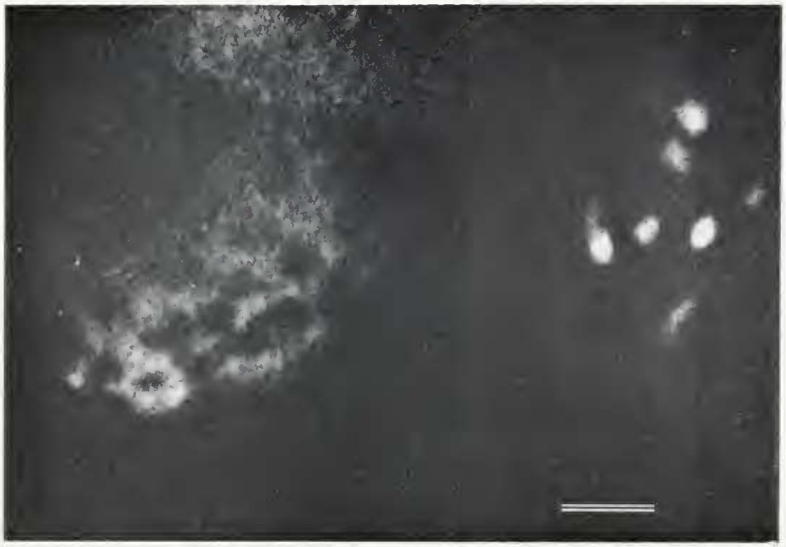

FIGURE 13. Glow and flash spots in the same organ. Glowing area (left) appeared after removal of the cuticle from half of the light organ. Microsources appear in a submaximal flash in the unskinned portion of the same light organ. Sizes of the spots range from 20 to 40 μ; diameters of the light rings (glowing rosettes) average about 70 μ. Calibration bar is 100 μ.

That the 21.6 μ microsource is actually a physiological unit is supported by the appearance of such a light source in three different conditions: (1) before and after an organ flash (Fig. 3); (2) in submaximal flashes (as discrete units; Fig. 4); and (3) during scintillation (Figs. 7, 8).

Previous workers in the field have generally considered the rosette to be the smallest physiological functional unit, perhaps partly because the histological data show photocytes highly organized around cylinders which act as distribution points for tracheae and nerves. However, Buck (1966) reported that luminous units of two sizes were observed visually: "glowing rings," and smaller "points" which compose the rings. Buck further reported that the glowing rings tend to be involved in the normal, widespread and coordinated responses, whereas disorganized, local and frenetic excitation patterns include an increasing proportion of the smaller units. Extending Buck's observations, the data in the present paper indicate that microsources are activated individually, even in normal flashes (Fig. 3). Adjacent microsources may be activated simultaneously to produce elongate spots or parts of rosettes (Fig. 9). Thus, if the activation of microsources making up a rosette were coordinated within the temporal resolution of the eye, the glowing ring would be observed as a functional unit. Buck's observations that this is the *normal* case implies that preferred innervation patterns exist within the ganglion which simultaneously excite all the axons innervating the microsources of a rosette.

The system for the control of light emission by the unit sources has not yet been fully elucidated. The morphological data of Smith (1963) show that

the nerve terminates in a complex "end organ" which abuts several (2–4) photocytes. From these end organs, the tracheoles bifurcate or trifurcate and, wrapped in extensions of the tracheolar cell, penetrate between the photocytes. Smith suggested that upon arrival of the nerve impulse, the excitation of the photocytes could occur by (1) the diffusion of neural chemicals through the end cell into neighboring photocytes; or (2) the secondary depolarization of the tracheolar cells which might then be channeled between the photocytes. In either case, a single nerve ending could control the activity of two to four adjacent photocytes. This inference is strongly supported by the physiological demonstration in the present paper that the unit source is two or three photocytes.

Although the data suggest that the photocyte pair (or trio) is the unit of function, it may not be the smallest. Smaller units having very short durations were not rigorously excluded by this study, since spots smaller than 10 μ are not easily differentiated from the noise of the image tube unless the spots recur from frame to frame. Smith (1963) reports photocyte granules *ca.* 2.5 μ in diameter. Such would not have been detected in the present study as discrete units of light emission, particularly if their duration is comparable to a shutter cycle (16 msec.) and thus would have appeared in only one frame. Since Buck (1966) showed photomultiplier recordings of some light emitting sources lasting only 20 msec, this is a possibility.

Coordination of light emission

The observation that the two light organs usually but not invariably light in coincidence confirms similar reports of visual impressions (*e.g.* Buck, 1966). However, the apparent variability of this occurrence (Fig. 2) suggests that separate nerve pathways from the central nervous system control the sixth and seventh segmental light organs.

The lack of complete coordination of the unit sources in producing a mass flash is evident in Figure 3–6. Some variability in synchronization of activation was observed, as flashes with differing rates of rise were observed. Another illustration of the degree of coordination of the microsources is that the durations of the organ flashes are considerably longer than the mean durations of the spots (Table I). Thus, the time spread of the organ flash is partly due to variability in microsource durations (see Table I). In reference to the question posed in the introduction, then, the organ flash is certainly the integration of briefer, staggered responses of a population of units which have activities of variable duration.

The shape of the decay phase of the light emission curve may be due to more than just the lack of new microsources turning on and the decay in intensity of the emitting ones. The fact that the average spot duration decreases with time (Table II) suggests the existence of an active turn-off mechanism. This would be in agreement with the conclusions of Buck and Case (1961) from electrophysiological data. However, another possibility is that units which appear late in the flash receive a lower activation stimulus or have a higher threshold which is manifest as a later onset of activity and a shorter duration.

The recurrence of the same spots from flash to flash indicates that the effectors are capable of activity in each successive flash. This shows that the refractory

time for each microsource is no greater than the minimum interflash interval (about 300 msec in this series of spontaneous flashes) and may be considerably less, as suggested by the data that some spots reappear in the same flash after once extinguishing. If so, the same photocytes may participate in each of the triple flashes that is the hallmark of the field behavior of males of some photurids.

Scintillation

In contrast to the partially coordinated responses of microsources making up the dim flashes, the microsources in scintillation appear to "sparkle" throughout the light organ. This phenomenon has been described by Case and Buck (1963) and also Buck (1966) who suggested that scintillation in response to eserine results from periodic "uncoordinated single unit firing". These observations are extended by statistical analyses presented here showing the asynchrony is not complete and that periodicity does occur. Case and Buck (1963) also suggested that these small luminous sources comprise "both single photocytes and small aggregations". This is basically supported by the present work: aggregations are observed (Fig. 9), but single spots (Figs. 7, 8) provide most of the activity. Since scintillation spots are not of significantly different sizes from the flash spots (Table III), the suggestion that each single spot is probably a photocyte pair or trio is applicable to both flashing and scintillation.

The nature of the response of the single unit

Since the organ flash is the combined responses of many individual units, the question arises as to the nature of the contribution of each: do the units respond with a quantal or graded energy output? From the standpoint of comparative physiology, the situation is anologous to muscle: are the single units all-or-none, or graded?

Although not conclusive, the results shown in Tables I and II strongly suggest the latter. The disparity between the durations of the microsources during flashing (139 msec) *vs.* scintillation (81 msec), the marked decrease in duration of the microsources as the flash proceeds, and the differences exhibited by microsources in successive flashes (Fig. 6), all suggest that there is no characteristic quantal response. This corresponds to Buck's (1966) visual observations that the "individual point intensity is not all-or-none but can be graded".

The single unit response, then, is apparently more analogous to the slow muscle fibers than to the all-or-none fast muscle fibers. The firefly evidently controls its light output by modulating single unit response as well as by recruiting additional units. This may provide an explanation for some of the flashing behavior of fireflies in the field: male fireflies often reduce flash intensity when approaching the female in a mating signal exchange.

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Summary

1. Microphotographs were obtained of a firefly (*Photuris sp.*) light organ on cinema film at 64 frames per second with the aid of an image intensifier.

2. Analysis of the film indicates that the two light organs usually flash simultaneously, but cases of one organ leading by 32–48 msec are seen.

3. Asynchrony is also observed within the light organ during a flash. Submaximal flashes are comprised of small spots of light ("microsources") which turn on at various times throughout the flash. Their durations average 139 ± 3 (S.E.) msec (range 31–360 msec) compared with 300–425 msec for an organ flash. Microsources are of fairly uniform size and shape having an average diameter of 21.6 ± 0.3 μ (S.E.) and a range of 11–36 μ. Microsources of this size are also seen as the functional unit of uncoordinated emission displays known as scintillation. It is suggested that the microsource is the smallest physiological unit of function in the firefly light organ.

4. Comparisons of glowing organs with histological sections demonstrate that the center portions of the "rosettes" are dark, and the centrifugal elements of the rosettes (the photocytes) emit light.

5. Microsources (21.6 μ in diameters) are somewhat smaller than the measured length of photocytes (34 μ) but larger than the width (8–10 μ). Thus the functional unit is identified as the center portions of 2 or 3 adjacent photocytes.

6. The whole organ flash is the integration of briefer staggered responses of a population of these single units which have activities of variable duration and intensities.

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